required fee for this extension of time, or any other fee occasioned by this paper, or to credit any overpayment in such fees, to Deposit Account No. 50-0320.

AMENDMENT

Please amend the application without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows.

Please amend the application without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows.

In the Claims

1-68. (Cancelled).

69. (Previously Presented) A diagnostic kit comprising at least one isolated and purified polypeptide comprising a WNV NS5 protein having ~~an~~ native conformation or non-denatured structure whereby the WNV NS5 protein is specifically reactive with antibodies against WNV but not substantially cross-reactive with antibodies against JEV, SLEV, or DENV.

70. (Previously Presented) The kit according to claim 69, wherein WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

71. (Previously Presented) The kit according to claim 69, wherein the WNV NS5 protein is an immunogenic fragment thereof.

72. (Previously Presented) The kit according to claims 69, wherein said NS5 protein is part of a fusion protein.

73. (Previously Presented) The kit according to claim 72, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said NS5 protein.

74. (Currently Amended) A method for detecting a WNV infection in a subject suspected of having said infection comprising the steps of (a) contacting a biological sample from the subject with an isolated and substantially purified polypeptide comprising a WNV NS5 protein having a native conformation or non-denatured structure whereby the NS5 protein is specifically reactive with anti-WNV antibodies ~~but not substantially cross-reactive with antibodies against JEV, SLEV, or DENV~~, and (b) detecting anti-WNV antibodies that have reacted with the WNV NS5 protein, wherein detection of the anti-WNV antibodies indicates a WNV infection.

75. (Cancelled)

76. (Previously Presented) The method of claims 74, wherein said NS5 protein is encoded by nucleic acid positions 7,633-1,377 of SEQ ID NO. 1.

77. (Previously Presented) The method 0 of claims 74, wherein the WNV NS5 protein is SEQ ID NO. 8.

78. (Previously Presented) The method of claim 74, wherein said NS5 protein is part of a fusion protein.

79. (Previously Presented) The method according to claim 78, wherein the fusion protein comprises a maltose binding protein or thioredoxin and said NS5 protein.

80. (Currently Amended) A method for detecting a first antibody to a WNV from a biological specimen of a subject suspected of being infected by said WNV comprising the steps of:

(a) contacting the biological specimen with a substantially pure WNV NS5 protein having a native conformation and non-denatured structure under conditions to form a complex between the NS5 protein and the first antibody, if present, that recognizes and binds the NS5 protein,

(b)detecting the first antibody of said complex, ~~wherein the NS5 protein is not substantially cross-reactive to an antibody against JEV, SLEV, or DENV~~.

81. (Original) The method according to claim 80, wherein said NS5 protein is coupled to a microsphere, adsorbed to nitrocellulose paper, or dried to nitrocellulose paper.

82. (Original) The method according to claim 80, wherein step (b) comprises the steps of:

(b_i) contacting said complex between said NS5 protein and said first antibody with a second antibody reactive against said first antibody,

(b_{ii}) detecting the second antibody,

wherein detecting the second antibody infers detecting the first antibody.

83. (Original) The method according to claim 82, wherein the second antibody includes a fluorescent marker or the second antibody is bound to colloidal gold or polystyrene microspheres.

84. (Original) The method according to claim 82, wherein the step of detecting the second antibody further comprises the step of immunofluorescence detection.

85. (Original) The method according to claim 82, wherein the second antibody is coupled to an enzyme which can be assayed.

86. (Original) The method according to claim 85, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

87. (Original) The method according to claim 80, wherein the biological specimen is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

88. (Original) The method according to claim 87, wherein the biological specimen is from a human.

89. (Previously Presented) A method according to claim 80, wherein the NS5 protein is a fusion protein.

90. (Previously Presented) A method according to claim 89, wherein said fusion protein comprises a maltose binding protein or thioredoxin and WNV NS5.

91. (Currently Amended) A method for rapidly detecting an anti-WNV antibody comprising the steps of:

(a) contacting a biological sample with a microsphere suspension, each microsphere coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against WNV ~~but not substantially cross-reactive with antibodies against JEV, SLEV, or DENV~~,

(b) incubating the microsphere suspension under conditions sufficient to promote the binding of an anti-WNV antibody to the NS5 proteins,

(c) contacting the microsphere suspension with a detection reagent capable of detecting the anti-WNV antibody,

(d) detecting the detection reagent,

wherein detection of the detection reagent indicates the presence the anti-WNV antibody in the biological sample.

92. (Original) The method according to claim 91, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

93. (Original) The method according to claim 92, wherein the biological sample is 10-20 microliters.

94. (Previously Presented) The method according to claim 91, wherein the step of incubating the microsphere suspension is performed under conditions sufficient to enhance reaction kinetics, wherein the conditions comprise incubating at 37°C, for about 30 minutes while keeping the microsphere suspension in motion.

95. (Original) The method according to claim 91, wherein the detection reagent comprises a antibody coupled to a fluorescent tag.

96. (Original) The method according to claim 91, wherein the detection reagent comprises a antibody coupled to an enzyme.

97. (Original) The method according to claim 96, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

98. (Original) The method according to claims 95, wherein step (d) comprises the step of immunofluorescence detection of said fluorescent tag of said antibody of said detection reagent.

99. (Currently Amended) A method for detecting a WNV infection in a biological specimen comprising the steps of:

(a) obtaining a suspension of microspheres each coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure wherein the WNV NS5 protein is specifically reactive with anti-WNV antibodies ~~but not substantially cross-reactive with antibodies against JEV, SLEV, or DENV~~;

(b) performing a microsphere immunoassay;

(c) obtaining a result indicating either the presence or absence of an anti-WNV antibody,

wherein the presence of an anti-WNV antibody indicates a WNV infection.

100. (Previously Presented) The method according to claim 99, wherein the microsphere immunoassay is a Luminex-based test or flow cytometer-based test.

101. (Original) The method according to claim 99, wherein the microsphere immunoassay is a lateral flow test.

102. (Original) The method according to claim 99, wherein the microsphere immunoassay is an agglutination test.

103. (Original) The method according to claim 99, wherein the microsphere immunoassay is a strip test.

104. (Original) The method according to claim 99, wherein the microsphere immunoassay is automated.

105. (Previously Presented) A method for the transfer of information comprising the steps of:

(a) carrying out the method of any of claims 74, 75, 80, 91, or 99 to obtain a result;

(b) providing the result to a third party.

106-125. (Cancelled).

126. (Currently Amended) A method for ~~rapidly detecting~~ rapid detection of an anti-WNV antibody in an animal by using an ELISA comprising the steps of:

(a) contacting a biological sample comprising at least one anti-WNV antibody with a reaction well surface, each reaction well surface coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each NS5 protein is specifically reactive to antibodies against WNV ~~but not substantially cross-reactive with antibodies against JEV, SLEV, or DENV~~,

(b) incubating the biological sample under conditions sufficient to promote the binding of the at least one anti-WNV antibody to the NS5 protein,

(c) contacting the reaction well surface with a detection reagent capable of detecting the anti-WNV antibody,

(d) detecting the detection reagent, wherein detection of the detection reagent indicates the presence of the at least one anti-WNV antibody in the biological sample.

127. (Currently Amended) The method according to claim 126, wherein the detection of the at least one anti-WNV antibody is indicative of a ~~recent~~ WNV infection.

128. (Previously Presented) The method according to claim 126, wherein the biological sample is from a previously WNV-vaccinated animal and wherein detection of the detection reagent indicates a recently sustained exposure of the previously WNV-vaccinated animal to WNV.

129-144. (Cancelled)

145. (Currently Amended) A method for ~~rapidly detecting~~ rapid detection of a recent or ongoing WNV infection in an animal susceptible of infection by said WNV, comprising the steps of:

(a) contacting a biological sample of said animal comprising anti-WNV antibodies with a microsphere suspension, each microsphere coupled to a substantially pure WNV NS5 protein having a native conformation or non-denatured structure whereby each WNV NS5 protein is reactive to said anti-WNV antibodies but not substantially cross-reactive to antibodies against JEV, SLEV, or DENV,

(b) incubating the microsphere suspension under conditions sufficient to promote the binding of said anti-WNV antibodies to the WNV NS5 protein,

(c) contacting the microsphere suspension with a detection reagent capable of detecting the anti-WNV antibody,

(d) detecting the detection reagent, wherein detection of the detection reagent indicates the presence of said anti-WNV antibody in said biological sample thereby detecting a recent or ongoing WNV infection.

146-155. (Cancelled).

156. (Previously Presented) A method for immunochromatographically testing for a WNV infection in an animal susceptible to said infection, comprising the steps of:

(a) contacting a biological sample comprising anti-WNV antibodies with a suspension of WNV NS5-coated microspheres to form a reaction mixture under conditions sufficient to promote binding of the anti-WNV antibodies to the WNV NS5-coated microspheres,

(b) placing the reaction mixture at the proximal end of the membrane strip having a proximal end, a distal end, and a plurality of zones each comprising secondary antibodies coupled thereto,

(c) incubating the membrane strip under sufficient conditions to promote the movement of the reaction mixture towards the distal end, said conditions being also sufficient to promote the binding of the microparticles to said secondary antibodies coupled to the membrane strip vis-à-vis interactions between said secondary antibodies and the anti-WNV antibodies,

(d) washing from the membrane strip any unbound microparticles, and

(e) detecting bound microparticles, wherein bound microparticles indicates a WNV infection in said animal.

157-161. (Cancelled).

162. (Previously Presented) The method for detecting a WNV infection according to claim 74, wherein the method is performed as a microsphere immunoassay, an agglutination assay, a slide test, a lateral flow test, an immunochromatographic assay, a fluorescence-based assay, a flow cytometric-based assay, or a Luminex-based assay.

163. (Previously Presented) The method for detecting a WNV infection according to claim 162, wherein the method is performed in less than about 3 hours.

164. (Previously Presented) The method for detecting a WNV infection according to claim 74, wherein the WNV NS5 protein is an immunogenic fragment thereof.

165. (Previously Presented) The method for detecting a WNV infection according to claim 74, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

166. (Previously Presented) The method for detecting a WNV infection according to claim 74, wherein the biological sample is from an animal, a human, a bird, a wild bird, a horse, a mouse, a cat, or a dog.

167. (Previously Presented) The method for detecting a first antibody to a WNV according to claim 80, wherein the method is performed as a microsphere immunoassay, an agglutination assay, a slide test, a lateral flow test, an immunochromatographic assay, a fluorescence-based assay, a flow cytometric-based assay, or a Luminex-based assay.

168. (Previously Presented) The method for detecting a first antibody to a WNV according to claim 167, wherein the method is performed in less than about 3 hours.

169. (Previously Presented) The method for detecting a first antibody to a WNV according to claim 80, wherein the WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

170. (Previously Presented) The method for detecting a WNV infection according to claim 80, wherein the WNV NS5 protein is an immunogenic fragment thereof.

171. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody according to claim 91, wherein the method is performed as a microsphere

immunoassay, an agglutination assay, a slide test, a lateral flow test, an immunochromatographic assay, a fluorescence-based assay, a flow cytometric-based assay, or a Luminex-based assay.

172. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody according to claim 171, wherein the method is performed in less than about 3 hours.

173. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody according to claim 91, wherein the biological sample is from an animal, a human, a bird, a wild bird, a horse, a mouse, a cat, or a dog.

174. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody according to claim 91, wherein the WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

175. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody according to claim 91, wherein the WNV NS5 protein is an immunogenic fragment thereof.

176. (Previously Presented) The method for detecting a WNV infection according to claim 99, wherein the method is performed as a microsphere immunoassay, an agglutination assay, a slide test, a lateral flow test, an immunochromatographic assay, a fluorescence-based assay, a flow cytometric-based assay, or a Luminex-based assay.

177. (Previously Presented) The method for detecting a WNV infection according to claim 176, wherein the method is performed in less than about 3 hours.

178. (Previously Presented) The method for detecting a WNV infection according to claim 99, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

179. (Previously Presented) The method for detecting a WNV infection according to claim 99, wherein the biological sample is from an animal, a human, a bird, a wild bird, a horse, a mouse, a cat, or a dog.

180. (Previously Presented) The method for detecting a WNV infection according to claim 99, wherein the WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

181. (Previously Presented) The method for detecting a WNV infection according to claim 99, wherein the WNV NS5 protein is an immunogenic fragment thereof.

182. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 126, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

183. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 126, wherein the biological sample is from an animal, a human, a bird, a wild bird, a horse, a mouse, a cat, or a dog.

184. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 126, wherein the WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

185. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 126, wherein the NS5 protein is a fusion protein.

186. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 185, wherein said fusion protein comprises a maltose binding protein or thioredoxin and WNV NS5.

187. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA as in any one of claims 126, wherein the step of incubating the biological sample is performed under conditions sufficient to enhance reaction kinetics, wherein the conditions comprise incubating at 37°C for about 30 minutes while keeping the biological sample in motion.

188. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 126, wherein detection reagent comprises an antibody coupled to a fluorescent tag.

189. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 126, wherein the detection reagent comprises an antibody coupled to an enzyme.

190. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 189, wherein the enzyme is selected from the group consisting of an oxidase, luciferase, peptidase, protease, glycosidase and phosphatase.

191. (Previously Presented) The method for detecting a WNV infection according to claim 126, wherein the WNV NS5 protein is an immunogenic fragment thereof.

192. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of an anti-WNV antibody in an animal by using an ELISA according to claim 188, wherein step (d) comprises the step of detecting the fluorescent tag of said antibody of said detection reagent by immunofluorescence.

193. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a ~~recent or ongoing~~ WNV infection according to claim 145, wherein the method is performed as a microsphere immunoassay, an agglutination assay, a slide test, a lateral flow test, an immunochromatographic assay, a fluorescence-based assay, a flow cytometric-based assay, or a Luminex-based assay.

194. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a ~~recent or ongoing~~ WNV infection according to claim 145, wherein the method is performed in less than about 3 hours.

195. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a ~~recent or ongoing~~ WNV infection according to claim 145, wherein the biological sample is selected from the group consisting of bodily fluid, blood, serum, plasma, saliva, tears, feces, semen, mucous, tissue, tissue homogenate, cellular extract, and spinal fluid.

196. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a ~~recent or ongoing~~ WNV infection according to claim 145, wherein the biological sample is from an animal, a human, a bird, a wild bird, a horse, a mouse, a cat, or a dog.

197. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a ~~recent or ongoing~~ WNV infection according to claim 145, wherein the WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

198. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a recent or ongoing WNV infection according to claim 145, wherein the WNV NS5 protein is a fusion protein.

199. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a recent or ongoing WNV infection according to claim 145, wherein the WNV NS5 protein is an immunogenic fragment thereof.

200. (Currently Amended) The method for ~~rapidly detecting~~ the rapid detection of a recent or ongoing WNV infection according to claim 198, wherein said fusion protein comprises a maltose binding protein or thioredoxin and WNV NS5.

201. (Previously Presented) The method for immunochromatographically testing for a WNV infection according to claim 156, wherein the WNV NS5 protein is SEQ ID NO. 8 or is encoded by nucleic acid positions 7,633-10,377 of SEQ ID NO. 1.

202. (Previously Presented) The method for for immunochromatographically testing for a WNV infection according to claim 156, wherein the method is performed in less than about 3 hours.